PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of:

MUNROE et al.

Group Art Unit: 1647 (expected)

Application No.: Not yet assigned (continuation of 09/222,995)

Examiner: R. Deberry (expected)

Filed: February 28, 2002

Attorney Dkt. No.: 108074-00023

For: AN ISOLATED HUMAN EDG-4 RECEPTOR (AS AMENDED)

PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, D.C. 20231

February 28, 2002

Sir:

Prior to initial examination of the application, please amend the above-identified application as follows:

IN THE TITLE:

Please amend the title to read as follows.

--AN ISOLATED HUMAN EDG-4 RECEPTOR--.

IN THE ABSTRACT:

Please add the Abstract attached to this Response on a separate sheet.

IN THE SPECIFICATION

Please amend the specification as follows: A marked up copy of the specification is attached hereto.

Page 46, line 15 (irrespective of printed line numbers), after "GGA-3']", please insert -(SEQ ID NO: 1)-.

Page 47, line 13 (irrespective of printed line numbers), after "-3']", please insert -(SEQ ID NO: 2)-.

Page 47, line 14 (irrespective of printed line numbers), after "-3']", please insert -(SEQ ID NO: 3)-.

Page 47, line 16 (irrespective of printed line numbers), after "-3']", please insert -(SEQ ID NO: 4)-.

Page 47, line 17 (irrespective of printed line numbers), after "-3']", please insert -(SEQ ID NO: 5)-.

Page 49, line 3 (irrespective of printed line numbers), after "-3']", please insert -(SEQ ID NO: 6)-.

Page 49, line 4 (irrespective of printed line numbers), after "-3']", please insert -(SEQ ID NO: 7)-.

Page 50, line 8 (irrespective of printed line numbers), after "-3']", please insert -(SEQ ID NO: 8)-.

Page 50, line 9 (irrespective of printed line numbers), after "-3']", please insert -(SEQ ID NO: 9)-.

Page 50, line 10 (irrespective of printed line numbers), after "-3']", please insert -(SEQ ID NO: 10)-.

Page 62, line 5 (irrespective of printed line numbers), after "-3']", please insert -(SEQ ID NO: 11)-.

Page 62, line 7 (irrespective of printed line numbers), after "-3']", please insert -(SEQ ID NO: 12)-.

Please insert new pages 1-16 containing the Sequence Listing at the end of the application.

IN THE CLAIMS:

Please add the following new claims.

--35. An isolated EDG receptor that upon activation results in increased induction of IL-8 or NF-κB, wherein said EDG receptor is a human EDG-4 receptor having the amino acid sequence of SEQ ID NO: 17 or SEQ ID NO: 22, or a human EDG-4 receptor variant having an amino acid sequence which is at least 91% identical to SEQ ID NO: 17 or SEQ ID NO: 22, with the proviso that said human EDG-4 receptor variant is not a rat EDG receptor.

36. The isolated EDG receptor of claim 35, wherein said EDG receptor is activated by a lysolipid selected from one or more of the group consisting of lysophosphatidic acid (LPA), sphingosine-1-phosphate (S1P) and sphingosine phosphocholine (SPC).

37. The isolated EDG receptor of claim 35, wherein said EDG receptor has the amino acid sequence of SEQ ID NO: 17.

38. The isolated EDG receptor of claim 35, wherein said EDG receptor has the amino acid sequence of SEQ ID NO: 22.

39. An isolated EDG receptor that upon activation results in increased induction of IL-8 or NF-κB, wherein said EDG receptor is a human EDG-4 receptor having the

amino acid sequence of SEQ ID NO: 17 or SEQ ID NO: 22, or a human EDG-4 receptor variant having an amino acid sequence which is at least 91% identical to SEQ ID NO: 17 or SEQ ID NO: 22, with the proviso that said human EDG-4 receptor variant has an amino acid other than phenylalanine at position 273 according to Figure 17B.

40. The isolated EDG receptor of claim 39, wherein said EDG receptor is activated by a lysolipid selected from one or more of the group consisting of lysophosphatidic acid (LPA), sphingosine-1-phosphate (S1P) and sphingosine phosphocholine (SPC).

41. An isolated EDG receptor that upon activation results in increased induction of IL-8 or NF-κB, wherein said EDG receptor is a human EDG-4 receptor having the amino acid sequence of SEQ ID NO: 17 or SEQ ID NO: 22, or a human EDG-4 receptor variant having an amino acid sequence which is at least 99% identical to SEQ ID NO: 17 or SEQ ID NO: 22.

42. The isolated EDG receptor of claim 41, wherein said EDG receptor is activated by a lysolipid selected from one or more of the group consisting of lysophosphatidic acid (LPA), sphingosine-1-phosphate (S1P) and sphingosine phosphocholine (SPC).

43. The isolated EDG receptor of claim 41, wherein said human EDG-4 receptor variant has an amino acid other than phenylalanine at position 273 according to Figure 17B.--

REMARKS

In this amendment, applicants amend the specification by inserting a Sequence Listing as new pages 1-16 at the end of the application and by labeling the sequences in the specification consistent with the Sequence Listing.

Please charge any fee deficiency or credit any overpayment to Deposit Account No. 01-2300.

Respectfully submitted,

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ABSTRACT OF THE DISCLOSURE

A novel, isolated EDG receptor that upon activation results in increased induction of IL-8 or NF-κB. Preferably, the EDG receptor is a human EDG-4 receptor, which has an amino acid sequence shown in Figures 16A and 16B, or a variant of these sequences having at least 91% sequence identity.

13B). Therefore, induction of inflammatory gene expression pathways is a conserved feature of EDG-4 in humans and rats, and likely reflects a fundamental biological aspect of receptor function.

Together, these results suggest that the SRE response is a shared feature of many different edg/lysolipid receptors, and can be used to verify the response of intact, functional receptors to their cognate agonist(s). On the other hand, the NF-κB response is shared by a subset of edg/lysolipid receptors which are specialized to mobilize inflammatory gene expression and immune system recruitment. Since EDG-1, EDG-3, EDG-4 and EDG-7 are all S1P/SPC receptors, their varying and even overlapping tissue distribution and inducibility frustrate the meaningful design, screening and therapeutic testing of anti-inflammatory S1P analogs unless the subtype specificity of inflammatory signaling is appreciated. This complexity highlights the value and utility of the recombinant inflammatory lysolipid receptors and the functional assays specified herein.

EXAMPLE 12. Identification of human expressed sequence tags (ESTs) homologous to rat H218 (EDG-4).

A BLAST search of the complete GenBank database was conducted with the sequence of an oligonucleotide RE4_181F [3'-GAGAAGGTTCAGGAACACTACAATTACACCAA GGA-3'] (SEQ ID NO:1), based on the sequence of rat EDG-4. The search identified a human EST (GenBank accession AA804628), which was 88% identical to the corresponding region of rat EDG-4 cDNA (GenBank accession U10699). A subsequent TBLASTN search of the EST database using the predicted polypeptide product of the rat EDG-4 cDNA (according to accession number U10699) revealed 2 other matching EST's (accession AA827835 and AA834537) in addition to the original human EST. The 3 EST's encompassed the predicted translation start site of human EDG-4 (based on similarity to rat EDG-4), overlapped each other extensively, and together spanned some 109 codons of the N-terminal portion of the human EDG-4 polypeptide (Figure 14). The predicted fragment of the human EDG-4 polypeptide showed 90.1% identity and 93.3% similarity to the equivalent fragment of rat EDG-4, suggesting the human polypeptide is an ortholog of the rat EDG-4 gene product, rather than a closely related gene product. A BLAST search was then conducted with the complete sequence of rat EDG-4 cDNA (accession number U10699) against the EST database. In addition to the previously identified EST's, 2 EST's apparently derived from the 3'-untranslated region of human EDG-4 cDNA adjacent to the

poly(A) tail were found (AA767046 and N93714). Of the 5 human EST's identified in total, only N93714 was present in the public database before February 19, 1998. This EST was derived from the 3' end of a 1421 bp cDNA insert which contained no coding region. The closest match recorded in the DBEST database entry (accession 500502) was a cGMP phosphodiesterase. The 5' end of the clone had been sequenced and given the GenBank accession W21101; however, similarity to other cDNAs was obscured by the presence of an Alu sequence.

EXAMPLE 13. Survey of potential cDNA sources using 5' end and 3' end diagnostic PCR.

To evaluate possible sources of human EDG-4 cDNA from HeLa cells (which express the inflammatory S1P/SPC receptor) and lung (a predominant site of EDG-4 expression in rat) for the presence of the desired cDNA fragments, diagnostic PCR primers were designed from the cluster of 5' end EST's (AA804628, AA834537 and AA827835) and 3' end EST's (N93714 and AA767046):

5' end primers:

HE4-DF1 [5'-ATTATACCAAGGAGACGCTGGAAAC-3'] (SEQ ID NO:2)

HE4-DR1 [5'-AGAGAGCAAGGTATTGGCTACGAAG-3'] (SEQ ID NO:3)

3' end primers:

HE4-DF2 [5'-TCCTCTCCTCGTCACATTTCCC-3'] (SEQ ID NO.4)

HE4-DR2 [5'-GCATTCACAAGAAATTACTCTGAGGC-3'] (SEQ ID NO:5)

Template sources: 1) cDNA library from WI-38 lung fibroblasts (Origene Technologies Inc., Cat. DLH-102); 2) cDNA library from human lung (Clontech, Cat. 7114-1); 3) cDNA library from HeLa cells (Invitrogen, Cat. A550-26); 4) First strand cDNA prepared in-house from HeLa cell total RNA. Each template was amplified with each pair of primers using the Expand™ PCR system from Boehringer Mannheim (Cat.1681-842).

Each reaction contained the following reagents:

2 µl	10x PCR Buffer 3
0.4 µl	25mM dNTP mix
0.6 µl	Primer HE4-DF1 or HE4-DF2 (10 µM)
0.6 µl	Primer HE4-DR1 or HE4-DR2 (10 µM)

amplified, containing the complete coding region. These primers were used in a PCR reaction with the WI-38 human lung fibroblast cDNA library (Origene) as follows:

HE4-DF3 [5'-GAGCCCCACCATGGGCAGCTTGTACT-3'] (SEQ ID NO.6)
HE4-DR2 [5'-GCATTCACAAGAAATTACTCTGAGGC-3'] (SEQ ID NO.7)

Each reaction contained the following reagents:

5 µl	10x PCR Buffer 3
1.0 µl	25mM dNTP mix
1.5 µl	Primer HE4-DF3 (10 µM)
1.5 µl	Primer HE4-DR2 (10 µM)
0.75 µl	Expand™ enzyme (2 units)
39.25 µl	water
1 µl	cDNA template (250 ng or 500 ng of DNA)

PCR conditions:

Incubate:	94°C for 2 min
10 cycles:	94°C for 40 sec
	60°C for 40 sec
	68°C for 5 min
25 cycles:	94°C for 40 sec
	60°C for 40 sec
	68°C for 3 min
Incubate:	68°C for 8 min
Hold:	4°C

Amplified reactions from 250 ng (tube 227-45) and 500 ng (227-50) of cDNA template each contained 3 PCR products 2 kb or larger. The PCR reaction and the DNA fragments from the gel were purified using QIAquick PCR purification kit (Qiagen Cat. 28106) and QIAquick gel extraction kit (Qiagen, Cat. 28704), respectively. Diagnostic PCR reactions were carried out on each of the 3 PCR products, and all

3 yielded the expected diagnostic PCR products using both the 5' end and 3' end primer pairs. Because they differed in size (~2 kb, 2.2 and 2.4 kb) and yet amplified with primers from the translation start and the 3'-untranslated region, all 3 may represent different alternatively spliced edg-4 transcripts.

The 3 PCR products were used as templates to reamplify human edg-4 with primers containing restriction sites suitable for cloning into an expression vector. Two different 3'-end primers were selected with longer (HE4-DR3) or shorter (HE4-DR4) 3'-untranslated regions. The following PCR primers and PCR conditions were used:

HE4-DF4	[5'-TTTAAAAAGCTTCCCACCATGGGCAGCTTGTACT-3']	(SEQ ID NO: 8)
HE4-DR3	[5'-TATATATCTAGACATTCACAAGAAATTACTCTGAGGC-3']	(SEQ ID NO: 9)
HE4-DR4	[5'-TATATATCTAGAGGAAATGTGACGAGGAGAGG-3']	(SEQ ID NO: 10)

Each reaction contained the following reagents:

5 µl	10x PCR Buffer 3
1.0 µl	25mM dNTP mix
1.5 µl	Primer HE4-DF4 (10 µM)
1.5 µl	Primer HE4-DR3 or HE4-DR4 (10 µM)
0.75 µl	Expand ™ enzyme (5 units)
39.25 µl	water
1 µl	DNA

PCR conditions:

Incubate:	94°C for 2 min
28 cycles:	94°C for 40 sec
	60°C for 40 sec
	68°C for 3.5 min
Incubate:	68°C for 8 min
Hold:	4°C

A pair of primers was designed from two ends of reading frame of human edg-4 cDNA sequence to engineer the edg-4 open reading frame into a vector designed for GFP fusion protein expression, with the GFP tag carboxy-terminal to the full-length EDG-4 polypeptide:

5'-End Primer: Contains Site for Kpn I enzyme, and optimized (Kozak) translation initiation sequence:

HE4-ATG KpnF: [5'-TTTAAAGGTACCGCCACCATGGGCAGCTTGTAC-3'] (SEQ ID NO:11)

3'-End Primet: Contains site for XbaI enzyme, and lacks naturally-occurring edg-4 stop codon:

HE4-xba/1096R: [5'-TATATATCTAGAGACCACCGTGTTGCCCTCCAG-3'] (SEQ ID NO:12)

pc3-hedg4#36 plasmid DNA was amplified with the above pair of primers under the following conditions of PCR amplification, using the Expand™ PCR system from Boehringer Mannheim (Cat. 1681-842).

The reaction contained the following reagents:

5 µl of 10x PCR Buffer 3
1.0 µl of 25mM dNTP mix
1.5 µl of Primer HE4-ATG KpnF (10 pmol/l)
1.5 µl of Primer HE4-xba/1096R (10pmol/l)
0.75 µl of Enzyme (2 units)
39.25 µl water
1 µl DNA

PCR conditions:

Incubate:	94°C for 2 min
10 cycles:	94°C for 1 min
	50°C for 1 min

Figure 17B

Alignment of HEDG4 with pC3-hedg4#36 translation product and rat H218 (REDG4). Differences between pC3-hedg4#36 translation product and previously determined HEDG4 polypeptide are indicated in reverse text. Differences between rat and human edg-4 polypeptide sequences are shown in bold, shaded text.

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           1                                                     50
    HEDG4  MGSLYSEYLN PNKVQEHYNY TKETLETQET TSRQVASAFI VILCCAIVVE
 HEDG4#36  MGSLYSEYLN PNKVQEHYNY TKETLETQET TSRQVASAFI VILCCAIVVE
    REDG4  MGGLYSEYLN PEKVQEHYNY TKETLDMQET PSRKVASAFI IILCCAIVVE

           51                                                   100
    HEDG4  NLLVLIAVAR NSKFHSAMYL FLGNLAASDL LAGVAFVANT LLSGSVTLRL
 HEDG4#36  NLLVLIAVAR NSKFHSAMYL FLGNLAASDL LAGVAFVANT LLSGSVTLRL
    REDG4  NLLVLIAVAR NSKFHSAMYL FLGNLAASDL LAGVAFVANT LLSGPVTLSL

           101                                                  150
    HEDG4  TPVQWFAREG SAFITLSASV FSLLAIAIER HVAIAKVKLY GSDKSCRMLL
 HEDG4#36  TPVQWFAREG SAFITLSASV FSLLAIAIER HVAIAKVKLY GSDKSCRMLL
    REDG4  TPLQWFAREG SAFITLSASV FSLLAIAIER QVAIAKVKLY GSDKSCRMLM

           151                                                  200
    HEDG4  LIGASWLISL VLGGLPILGW NCLGHLEACS TVLPLYAKHY VLCVVTIFSI
 HEDG4#36  LIGASWLISL VLGGLPILGW NCLGHLEACS TVLPLYAKHY VLCVVTIFSI
    REDG4  LIGASWLISL ILGGLPILGW NCLDHLEACS TVLPLYAKHY VLCVVTIFSV

           201  I                                               250
    HEDG4  ILLAXVALYV RIYCVVRSSH ADMAAPQTLA LLKTVTIVLG VFIVCWLPAF
 HEDG4#36  ILLAVVALYV RIYCVVRSSH ADMAAPQTLA LLKTVTIVLG VFIVCWLPAF
    REDG4  ILLAIVALYV RIYFVVRSSH ADVAGPQTLA LLKTVTIVLG VFIICWLPAF

           251                                                  300
    HEDG4  SILLLDYACP VHSCPILYKA HYXFAVSTLN SLLNPVIYTW RSRDLRREVL
 HEDG4#36  SILLLDYACP VHSCPILYKA HYLFAVSTLN SLLNPVIYTW RSRDLRREVL
    REDG4  SILLLDSTCP VRACPVLYKA HYFFAFATLN SLLNPVIYTW RSRDLRREVL

           301                                                  350
    HEDG4  RPLQCWRPGV GVQGRRRGGT PGHHLLPLRS SSSLERGMHM PTSPTFLEGN
 HEDG4#36  RPLQCWRPGV GVQGRRRGGT PGHHLLPLRS SSSLERGMHM PTSPTFLEGN
    REDG4  RPLLCWRQGK GATG.RRGGN PGHRLLPLRS SSSLERGLHM PTSPTFLEGN

           351
    HEDG4  TVV~
 HEDG4#36  TVV~
    REDG4  TVV~
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